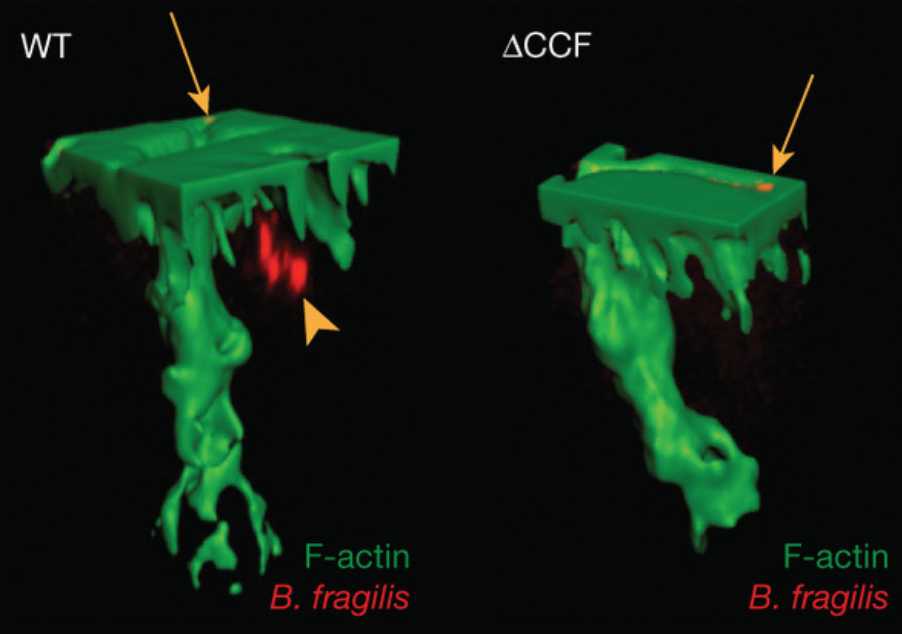


The gut is home to trillions of bacteria that play numerous roles in human health; understanding how the different bacterial species interact with each other and survive the harsh gut environment is important to therapeutics aimed at establishing healthy gut microbial communities. This image shows three-dimensional reconstructions of tiny pockets in the intestinal wall called colon crypts (green). (Left panel) While normal gut bacteria called *Bacteroides fragilis* (*B. fragilis*) (red) can be detected on the surface (arrow) and within the colon crypt (arrowhead) of mice, (Right panel) *B. fragilis* lacking "commensal colonization factors" can only be detected on the surface (arrow). As described in this chapter, these newly identified factors may help certain bacterial species establish themselves and survive in protective spaces within the gut.

*Image courtesy of Dr. Sarkis Mazmanian, California Institute of Technology. Adapted by permission from Macmillan Publishers Ltd: Nature, Lee SM, Donaldson GP, Mikulski Z, Boyajian S, Ley K, Mazmanian SK. Bacterial colonization factors control specificity and stability of the gut microbiota. 501:426-429, copyright 2013.*

# Digestive Diseases and Nutrition

*Digestive diseases are among the leading causes of doctor visits, hospitalizations, and disability in the United States each year. These conditions span a wide spectrum of disorders that affect the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. The latest concerted effort to address the burden of all digestive diseases combining multiple big data sources estimated a total of 72 million ambulatory care visits to physicians' offices, and hospital emergency and outpatient departments with a primary diagnosis of digestive diseases in the United States.[1] In addition, 4.6 million hospitalizations with a primary diagnosis of digestive diseases and 13.5 million hospitalizations with a primary or secondary diagnosis of digestive diseases were reported.[1] More recently, a study focusing specifically on the clinical and economic burden of emergency department visits reported 15.1 million visits with a primary diagnosis of digestive diseases and a total charge of $27.9 billion in 2007.[2]*

Some digestive diseases are common and others quite rare. Yet collectively, they strike individuals across the lifespan, exacting a significant toll on public health in terms of their effects on quality of life, years lost due to premature death, and costs associated with hospitalization and pharmaceutical and surgical interventions. NIDDK-supported scientists are vigorously pursuing research with the ultimate goal of reducing the public health burden associated with digestive diseases. Such efforts aim to better understand how widespread these diseases are across the United States and in specific population groups, to identify their causes and how they progress, and to test new interventions for prevention and treatment, including drugs, surgery, and behavior modification.

Inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis, are marked by damaging inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. These diseases often strike early in life, with a peak age of onset in adolescence or young adulthood. Treatment frequently requires prolonged use of multiple drugs and may require surgery, including removal of the affected region of the intestine. Scientists are investigating the complex interactions among the genetic, environmental, immune, microbial, and cellular factors that contribute to, or protect against, the development of IBD. The continued discovery of predisposing genetic variations, potential autoimmune and microbial influences, and new methods to repair damaged intestinal tissue will help catalyze the design of novel therapeutic strategies. Research on controlling intestinal inflammation has potential benefits not only for people with IBD, but also for those at risk of developing colorectal cancer.

Diseases of the stomach and intestines include some of the most common digestive diseases, such as peptic ulcer disease, which is typically caused by an infection with the bacterium *Helicobacter pylori* or use of non-steroidal anti-inflammatory drugs. Stomach and intestinal disorders also include functional bowel disorders, which result in symptoms of abdominal pain and altered bowel habits. For

[1] *Everhart JE and Ruhl CE. Burden of Digestive Diseases in the United States Part I: Overall and Upper Gastrointestinal Diseases. Gastroenterology 136: 376-386, 2009.*

[2] *Myer PA, et al. Clinical and Economic Burden of Emergency Department Visits Due to Gastrointestinal Diseases in the United States. Am J Gastroenterol 108:1496-1507, 2013.*

example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown. Gastroesophageal reflux disease, in which stomach acids rise up into the esophagus, is a common functional bowel disorder that can lead to a condition known as Barrett's esophagus. This condition, in which cells lining the esophagus turn into an intestinal type of cell, is associated with a heightened risk of esophageal cancer—one of the cancer types still on the rise in the United States. Gastroparesis, another type of functional bowel disorder, is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. While many cases of gastroparesis are of unknown origin, a common cause is diabetes, which is thought to damage nerves leading to the stomach and controlling movement of food. Sphincter of Oddi dysfunction is a disorder marked by attacks of abdominal pain and is often found in individuals who have had their gallbladders surgically removed. Fecal incontinence, or impaired bowel control, is another bowel disorder that poses a major public health burden. Although fecal incontinence is more common in older adults, it can affect people of any age. Because it is difficult to talk about, many people suffer without seeking professional treatment for this surprisingly prevalent condition. Researchers thus aim both to examine barriers in addressing fecal incontinence and to develop improved treatment strategies.

Some digestive diseases can be triggered by the body's reaction to certain foods. For example, in individuals with celiac disease, the immune system reacts to the protein gluten—a component of wheat, barley, and rye—and damages the small intestine. This damage interferes with the ability of the intestine to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, slower growth and short stature. The only current treatment for celiac disease is maintenance of a strict gluten-free diet, which is difficult for many people. Diagnosis of celiac disease can be challenging, due to the non-specific and often minimal symptoms in people with the disorder. Recent and continued advances in the understanding of genes that predispose individuals to develop celiac disease may contribute to improved diagnosis in the future through genetic-based screening.

The microorganisms that inhabit the GI tract, termed the gut "microbiome," are important factors in maintaining or tipping the balance between digestive health and disease. These microbes can affect long-term health and nutritional status in some surprising ways, depending on their interactions with each other, with intestinal cells, and with nutrients ingested by their human host. Scientists are gaining insights into the ways these GI microorganisms influence the development and function of the digestive tract and other systems throughout the body, such as those with immune and metabolic functions, as well as how the composition of the GI microbial community changes with factors such as age, geography, diet, and antibiotic usage.

The exocrine pancreas, which secretes enzymes required for digestion, is vulnerable to disorders such as acute and chronic pancreatitis and their complications. Common causes of pancreatitis include gallstones, heavy alcohol use, inherited genetic factors, and drugs. In all forms of pancreatitis, digestive enzymes attack the pancreas from within, causing inflammation, loss of function, and severe pain. Research has elucidated genetic and other factors contributing to pancreatitis that may lead to ways to treat or prevent this disorder.

The liver is an organ within the digestive system that performs many critical metabolic functions, including processing and distribution of nutrients such as fats. When the liver is functionally compromised by disease, serious adverse effects on health can occur, which sometimes leads to complete liver failure. Some liver diseases primarily affect children, such as biliary atresia (a progressive inflammatory liver disease), while others generally affect adults, such as a form of nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH). In recent

years, however, NAFLD has been increasingly diagnosed in children in the United States as well, concurrent with rising overweight and obesity. Some forms of liver disease are caused by viral infection such as hepatitis B and C, or by genetic mutations such as alpha-1-antitrypsin deficiency; others arise from diverse factors such as autoimmune reactions, drug toxicity, and other triggers, some of which are unknown. Many liver diseases, such as chronic hepatitis B and C, place individuals at elevated risk for developing liver cancer. A healthy liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. Because the number of livers available from deceased donors is limited, research is critical to identify liver disease early, find methods to preserve liver function in people with liver disease, and develop and further study new treatment options, including experimental, cell-based approaches to liver regeneration.

The number of Americans who are overweight or obese has risen dramatically in recent decades and is now at epidemic levels. Obesity is associated with numerous diseases, including type 2 diabetes, heart disease, and cancer. Multiple factors contribute to obesity. As scientists elucidate the molecular, genetic, microbial, and environmental factors that influence appetite, metabolism, and energy storage, they are identifying potential avenues for the development of new intervention strategies to promote safe, long-term weight loss. In addition to new pharmacologic interventions for obesity that may arise from research, existing bariatric surgical techniques are being evaluated for their long-term impacts on weight loss, obesity-associated disease, and well-being. Investigators are also continuing research to help people achieve healthy lifestyles that include physical activity and improved diet. (Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the Obesity chapter.)

Other nutrition-related disorders under investigation involve specific, inherited alterations in nutrient metabolism. NIDDK-supported research has enhanced knowledge of how these nutritional disorders develop and how they can best be treated.

## GUT MICROBIOME AND HEALTH

**A "Who's Who" of the Gut Microbial Community: Their Origins and Health Effects:** Researchers have used new and creative methods to identify specific bacterial strains from the microbial communities of humans, other organisms, and habitats on land and sea; learn how they colonize the gut; and determine their impacts on host health and disease. The human gut is home to an estimated 100 trillion bacterial cells, the composition of which varies greatly between individuals. Unraveling the mystery of which microbes are present in the gut, how they come to reside there, and their health implications for the host is the subject of recent investigations by one research group.

One study focused on the question of how microbes colonize the mammalian gut. Scientists used the inventive approach of testing how microbes from non-native sources compete for resources and space in the intestinal environment. The scientists transplanted male mice raised under sterile conditions with microbes from a wide range of sources, including the guts of humans, zebrafish, and termites; skin and tongue of humans; or from the soil or a marine estuary. They conducted successive stages of experimentation in which microbes were shared between the original transplanted mice and other mice, using transplantation of intestinal contents and/or co-housing in the same cage. Over a few weeks, they collected fecal samples from the mice and analyzed the genetic material to identify bacterial species present. In their primary set of experiments, they set up mixed groups of transplanted mice rooming together, with some surprising results. For example, one group living arrangement consisted of four mice—one colonized by soil microbes, a second by termite gut microbes, a third by zebrafish gut microbes, and a fourth without any microbes. In this setup, the gut microbes of the four mice quickly came to resemble one another, with species from the soil and zebrafish gut dominating early on; however, after a week, the soil sample-derived microbiome became predominant in all the cohabitating animals. A single type of soil bacteria, a previously unidentified *Ruminococcus*, proved particularly successful at seizing the opportunity to

colonize the mouse gut, likely aided by its ability to process multiple types of carbohydrates in the gut. In another experiment, mice were transplanted with gut microbes from humans living in three very different environments (urban United States, rural Malawi, and the Venezuelan state of Amazonas, with its large population of indigenous peoples). The mice were then co-housed to develop a roughly average mix of human microbes. The mice harboring a composite human gut microbial mix were then housed in a cage with both mice transplanted with a composite of mouse gut microbes and mice without any gut microbes. Early on, human gut microbes dominated, even in the guts of mice with native mouse gut microbes. After 4 days, the mouse microbes were starting to overtake the invaders from the human gut, though the human gut microbes remained detectable weeks later. These experiments help to define the "succession" of bacterial species as they come to colonize sequentially and compete with each other in the unique environment of the mammalian gut. In addition, the researchers also measured some of the molecules related to bacterial (and host) metabolism, such as carbohydrates, short chain fatty acids, and bile acids, which help these species succeed in outcompeting others in the gut.

Another research project took a new approach to the problem of conducting a census of gut bacteria and providing valuable insights into their health effects. The scientists used combinations of gut microbes harvested from human stool samples and tested them in male mice raised under sterile conditions to be free of any microbes. Two weeks after transplanting the human gut microbes into the mice, they measured increases in a type of immune cell that prevents inappropriate inflammation in the gut, but they also saw an increase in fat deposits (adiposity). Using one of the human donors' samples as a representative, the researchers sequenced the bacterial genomes present. They identified 17 unique bacterial strains that, when given to mice, showed effects on immune cells and adiposity similar to the effects of the initial bacterial transplants. To find out which specific combinations of bacterial strain subsets were responsible for these effects, the researchers gave 94 different combinations of the bacteria as well as single bacterial strains to the mice. They then measured immune cells, adiposity, and products of nutrient metabolism, such as bile acids, fatty acids, and amino acids, and compared the results to measurements of the same elements in control mice that remained bacteria-free. Through these experiments, they identified which bacteria, alone or in combination, promoted these immune and metabolic functions. For example, they found several bacterial strains were associated with increased adiposity, including five strains in the bacterial group *Bacteroides*, two strains of *Bacteroidetes*, and *Escherichia coli*. Many of the same *Bacteroides* strains were also associated with expansion of the population of immune cells called regulatory T cells in the intestine.

These studies add to the storm of new knowledge about the mammalian gut microbial community, in terms of understanding the succession of species during colonization and teasing out effects of individual bacterial strains. This work also provides a new means for scientists to identify which resident gut microbes are helping or hindering their human hosts, in terms of key health indicators such as immune function, nutrient metabolism, and fat mass. These methods could be used in the future to identify probiotics or prebiotics—beneficial bacteria or the nutrients they rely on—to enhance human health and limit disease.

*Seedorf H, Griffin NW, Ridaura VK, …Gordon JI. Bacteria from diverse habitats colonize and compete in the mouse gut. Cell 159: 253-266, 2014.*

*Faith JJ, Ahern PP, Ridaura VK, Cheng J, and Gordon JI. Identifying gut microbe-host phenotype relationships using combinatorial communities in gnotobiotic mice. Sci Transl Med 6: 220ra11, 2014.*

**Evaluating Treatments for Childhood Malnutrition Based on Changes in Gut Bacteria:** A team of U.S. and Bangladeshi scientists discovered that children who are malnourished do not harbor gut bacteria typical for their age, even several months after receiving a nutritional intervention. The persistence of this "immature" collection of gut bacteria may be a reason why the children do not grow well even after receiving nutrient-dense food. Moderate or

severe forms of malnutrition affect a large number of children living in developing countries, such as Bangladesh. This malnutrition typically develops very early in life, between 3 and 24 months. Therapeutic interventions, such as the use of either a specially prepared, nutrient-dense processed food or indigenous foods high in calories and protein, have short-term, but not lasting, effects in terms of sustaining healthy growth and development. The community of microbes inhabiting the gut is known to play an important role in assisting their human host with many important functions, such as extracting nutrients from the diet and facilitating new blood vessel formation in the intestine. Scientists wondered whether malnutrition early in life might disrupt the establishment of a healthy, diverse microbial community, thereby limiting the impact of subsequent nutritional interventions. To test this idea, they identified the different microbial species present in fecal samples collected from children living in an impoverished urban area of Dhaka, Bangladesh. Using a technique that distinguishes among different types of bacteria, they were able to identify which bacterial species were present. They first used this method to characterize the microbial makeup of a group of children showing healthy growth and development for their age living in this part of the world. They confirmed this model of a healthy, or "mature," microbial community in another group of local, healthy children. Then, they compared them to severely malnourished children who were treated in a hospital in Dhaka for a few weeks with either a standard ready-to-use therapeutic food or a locally produced combination of rice and lentils with other nutrients. While both nutritional interventions improved growth of the malnourished children, they remained underweight and below normal height compared to healthy children. Fecal samples taken from the malnourished children before, during, and after treatment revealed a persistent immaturity in their gut microbial communities, such that they lacked bacterial species typically found in well-nourished children of a similar age. These findings suggest new strategies that might be used to improve the treatment of childhood malnutrition by bolstering the health of the gut microbial community, such as through more prolonged dietary interventions or development of targeted probiotics.

*Subramanian S, Huq S, Yatsunenko T,…Gordon JI. Persistent gut microbiota immaturity in malnourished Bangladeshi children. Nature 510: 417-421, 2014.*

## UNDERSTANDING GUT-MICROBIOME INTERACTIONS

**How a Species of Bacteria Makes Its Home in the Gut:** Researchers have identified a group of microbial genes that allow a species of bacteria to take up residence in a certain area of a mouse's gut. In both mice and humans, the gastrointestinal tract, especially the colon, is home to hundreds of different species of bacteria that are important for digestive health. While several studies have examined how certain gut microbes can trigger or protect against the inflammation that leads to diseases such as Crohn's disease or ulcerative colitis, it is not clear how bacteria grow and survive in the turbulent environment of the gastrointestinal tract. It is also not certain how species of bacteria interact with each other within the gut—an important factor to consider when developing probiotic therapies with the intent of establishing a healthy gut microbial community.

Researchers attempted to answer these questions by examining how a species of bacteria found in large numbers in the gut of humans and other mammals, called *Bacteroides fragilis* (*B. fragilis*), colonizes the gastrointestinal tract in a mouse model. The scientists introduced *B. fragilis* into "germ-free" male and female mice raised under sterile conditions and allowed the bacteria to colonize the gut. When the scientists then introduced a similar yet different species of bacteria to these mice, both bacterial species were able to co-exist in the gut. However, when the scientists tried to give more *B. fragilis* to mice that already had this species, the newly introduced bacteria could not establish themselves. This led the scientists to conclude that *B. fragilis* prefers a certain limited area, or niche, in the gut, and once it saturates this area, then no more *B. fragilis* can settle there. To explore this idea, the scientists examined the linings of the guts in the mice. They found that *B. fragilis* preferred to grow within small pockets in the intestinal wall called crypts, which

may act as "rooms" that, once occupied, resist further colonization by additional *B. fragilis*. Using a genetic screen, the scientists were able to identify a set of genes that becomes activated when *B. fragilis* colonizes the gut. These genes are necessary for the bacteria to occupy the crypts and to exclude additional bacteria of the same species beyond a certain capacity. When these genes were deleted from the bacteria, the bacteria could no longer take up residence in the crypts or prevent other, normal *B. fragilis* from growing there. Bacteria missing these genes were also more susceptible to being cleared away by an antibiotic than wild-type bacteria, suggesting that the crypts may also serve a protective role for the microbes growing in them. This study provides insight into how certain species of bacteria may establish themselves and survive in the gut. Further research on how beneficial bacteria colonize the gut in humans may help in the design of probiotic or antibiotic therapies for treating digestive diseases.

*Lee SM, Donaldson GP, Mikulski Z, Boyajian S, Ley K, and Mazmanian SK. Bacterial colonization factors control specificity and stability of the gut microbiota. Nature 501: 426-429, 2013.*

**Microbial Secretion Regulates Intestinal Immune Function:** Research has uncovered one way in which beneficial microbes in the intestine support healthy immune function in their hosts—by releasing substances called sphingolipids that keep the activity of local immune cells in check. The "symbiotic" microbes in the human intestine, whose presence benefits both parties, include members of the *Bacteroidetes* phylum, such as *Bacteroides fragilis* (*B. fragilis*), which release unique molecules called sphingolipids. These molecules act on a type of host immune cell called the invariant natural killer T (iNKT) cell, the function of which is to quickly activate the immune system by releasing a flood of chemicals called cytokines. Researchers wondered whether sphingolipids produced by beneficial bacteria might protect against intestinal inflammation from an overactive immune system. They explored this idea in both male and female mouse models and in cells grown in laboratory culture. They raised mice to have either normal *B. fragilis* in their intestines or a mutated form of the bacteria that could not produce sphingolipids. Comparing these two groups of mice, those with normal *B. fragilis* had reduced numbers of iNKT cells. When these mice were later challenged with a chemical that causes colonic inflammation similar to human ulcerative colitis, those harboring the mutated bacterium developed more severe disease than mice colonized with the unaltered bacterium capable of releasing sphingolipids. Purifying the sphingolipids produced by these bacteria enabled the researchers to observe their direct effects on cells in culture: the sphingolipids dampened iNKT cell activation. When the researchers gave the colitis-inducing chemical to mice harboring the mutated bacterium and then treated these mice with purified sphingolipids at birth, they saw that the sphingolipids restored protection against colitis development later on in life. These studies show how early exposure to beneficial intestinal bacterial products can offer life-long protection against inappropriate immune activation and inflammation through regulating the activity of the host immune system. These bacterial products may be useful in treating autoimmune and allergic disorders that appear later in life.

*An D, Oh SF, Olszak T,…Kasper DL. Sphingolipids from a symbiotic microbe regulate homeostasis of host intestinal natural killer T cells. Cell 156: 123-133, 2014.*

**Gut Protein Punches Holes in Bacteria:** A protein produced by intestinal cells kills certain bacteria by creating small holes in the microbes' protective outer membranes. The human gut is home to trillions of bacteria that play an important role in digestive health. In some cases—such as inflammatory bowel disease—the body's immune system reacts inappropriately to these microbes, irritating the intestinal lining. One way the body avoids this unwanted response is to limit contact between bacteria and the intestinal wall by killing any bacteria that get too close to the intestinal lining. However, it was not certain how the body accomplishes this.

A team of scientists tackled this problem by investigating proteins secreted by intestinal cells that are able to kill bacteria. The researchers focused on a protein called "RegIIIα," which was previously found to be lethal to certain bacteria, although it was not certain how

the protein actually killed the microbes. Additionally, RegIIIα is produced by intestinal cells when bacteria come in close contact with the lining of the gut, which suggests that RegIIIα could be used as a defense against intruding microbes. While investigating how RegIIIα kills bacteria, the scientists found that adding it to bacteria allowed a traceable dye to diffuse into the bacterial cells, which meant RegIIIα had caused the bacteria's protective outer membrane to become leaky—something that can easily be fatal to most microbes. They saw the same leakiness when RegIIIα was added to artificial membranes made up of the same components that are in bacterial membranes. Other experiments showed that RegIIIα was creating tiny pores about one millionth of a millimeter in diameter in these membranes. The scientists used X-ray crystallography and electron microscopy to visualize the pores, and they found that each one was made up of six RegIIIα proteins that assemble to form a doughnut-shaped hole. This means that if a microbe gets too close to the intestinal wall, RegIIIα proteins produced by gut epithelial cells can insert themselves into the microbe, puncturing and killing it. The deadly effects of RegIIIα were not seen with all bacteria, however; some types of microbes have an extra chemical on their membrane surface that can protect them from this type of attack.

These findings show how the RegIIIα protein secreted by intestinal cells is part of an elaborate defense mechanism that helps create a bacteria-free buffer zone between gut bacteria and the intestinal wall, potentially preventing unwanted inflammatory reactions. Further research may shed light into how this system might break down during disease and, importantly, how it might be harnessed to prevent illness.

*Mukherjee S, Zheng H, Derebe MG,…Hooper LV. Antibacterial membrane attack by a pore-forming intestinal C-type lectin. Nature 505: 103-107, 2014.*

## INVESTIGATING GUT INFLAMMATION

**Origins of Gut Inflammation Found in Specific Cell Type:** Scientists have discovered that processes taking place within a particular intestinal cell type called the Paneth cell are linked to the development of inflammation in a portion of the small intestine in mice. The resulting inflammation is similar to that seen in a form of human Crohn's disease. Crohn's disease, a form of inflammatory bowel disease that can affect the small or large intestines, is thought to result from an interplay between genetic susceptibility factors, such as those that affect immune function, and environmental factors, such as gut microbes. For example, one genetic mutation associated with Crohn's disease occurs in the *ATG16L1* gene, which is linked to an important process called "autophagy," or "self-eating." Cells use autophagy to reduce cellular stress by degrading and recycling extraneous or malfunctioning components. This genetic mutation also causes dysfunction in Paneth cells, which are known primarily for protecting against harmful gut microbes by secreting antimicrobial molecules. Why autophagy or Paneth cell dysfunction might be important in Crohn's disease, however, was unknown.

A team of researchers delved into how problems with processes such as autophagy occurring in intestinal cells might relate to the development of gut inflammation. Working with a series of mouse models genetically engineered to lack certain proteins important for autophagy and other processes in their intestinal cells, the team showed that turning off both the *Atg16L1* gene and a gene involved in monitoring proper protein structures resulted in reduced autophagy and increased stress in Paneth cells. Additionally, these animals exhibited a severe, spontaneous form of inflammation in the small intestine that closely mimicked cases of human Crohn's disease. This study sheds new light on the key cellular and genetic factors, and processes such as autophagy, that are involved in intestinal inflammation, particularly in Crohn's disease occurring within the small intestine. These findings offer important clues that may lead to better diagnosis and management of this form of inflammatory bowel disease in the future.

*Adolph TE, Tomczak MF, Niederreiter L,…Blumberg RS. Paneth cells as a site of origin for intestinal inflammation. Nature 503: 272-276, 2013.*

**Studies Identify Factors Associated with Intestinal Inflammation:** In an effort to understand the causes of inflammatory bowel disease, scientists have identified several genes and types of bacteria that are associated with these diseases in the human gut. Inflammatory bowel diseases (IBD) include conditions such as Crohn's disease and ulcerative colitis, which are characterized by symptoms such as diarrhea, intense abdominal pain, and weight loss. While the exact causes of IBD are not known, inflammation in the gut is believed to result from an inappropriate reaction of the cells lining the intestine to some of the trillions of bacteria that inhabit the digestive tract. Genetics also plays a large role in the development of IBD, as there have been over 160 areas of the human genome that have been identified to contain risk factors. Despite these known links, however, it is not clear which bacteria, and which specific genes, are important for the initiation and development of IBD.

To address these questions, a group of researchers set out to identify IBD-specific changes in the bacterial community in the human gut, along with changes in gene activity within the gut cells. They focused on the area of the small intestine closest to the colon, called the ileum, which is believed to be a primary site where Crohn's disease originates. The researchers took biopsies from ilea of male and female children and adolescents who had either Crohn's disease or ulcerative colitis. They then identified the bacteria in the samples and analyzed the genes that were activated in the human ileal cells. They compared the results to biopsies from individuals who did not have IBD. The researchers found that those with Crohn's disease or ulcerative colitis had higher levels of a type of bacteria called Proteobacteria and an increase in the activity of a gene called *DUOX2*. They also found that people with Crohn's disease had lower amounts of a type of bacteria called Firmicutes and lower activity of a gene called *APOA1*. This means people with IBD have a particular microbial and genetic "signature" that could provide targets for improved diagnosis and therapy. This signature could also be studied further to better understand these diseases.

Another group of researchers attempted to identify the bacteria associated with IBD by determining which bacteria are coated with a type of "antibody" or immune protein, called IgA, that the body produces to protect itself from foreign substances. IgA is present in the mucus layer covering the inside of the intestines, where it attaches to disease-causing bacteria and neutralizes them. First, the researchers used a model of colitis with female and male mice to determine if the degree of IgA coating can identify bacteria causing the disease. They found that the mice with colitis had more IgA-coated gut bacteria compared to normal mice, and the most highly coated bacteria were the species that were causing colitis in these animals. Next, the researchers analyzed human fecal samples and found that, like the mouse model, individuals with IBD had higher levels of IgA-coated gut bacteria than healthy controls. When germ-free mice with chemically induced colitis were colonized with bacteria from the human samples, only the bacterial species that had been highly coated with IgA caused severe intestinal inflammation and bleeding. The bacteria that had been poorly coated with IgA had no effect. This means the amount of IgA coating on gut bacteria may pinpoint which species are most likely to trigger an inflammatory response, and targeting those bacteria may be an effective strategy for treating IBD.

Together, these studies shed light on the complicated origins of IBD. Similar studies may identify more factors that are involved, which could lead to new diagnostic tools and treatments for these painful and debilitating diseases.

*Haberman Y, Tickle TL, Dexheimer PJ,…Denson LA. Pediatric Crohn disease patients exhibit specific ileal transcriptome and microbiome signature. J Clin Invest 124: 3617-3633, 2014.*

*Palm NW, de Zoete MR, Cullen TW,…Flavell RA. Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. Cell 158: 1000-1010, 2014.*

## INSIGHTS INTO FUNCTIONAL BOWEL DISORDERS

**New Role in Bowel Function Discovered for Familiar Protein:** In a recent mouse study, scientists found that a well-known anti-cancer protein called

retinoblastoma 1, or RB1, plays a surprising role in gastrointestinal motility. Embedded in the lining of the mammalian gastrointestinal tract is a mesh-like system of nerves called the enteric nervous system. These nerves send signals to gut muscles to contract and relax in a synchronized fashion, pushing ingested food through the intestines and enabling stool to pass normally. Failure of the enteric nervous system to communicate with the intestinal muscles could lead to gastrointestinal motility disorders. For example, in people with Hirschsprung's disease, nerves are missing from portions of the intestine, so the gut muscles do not work properly, and the contents of the intestines become stuck. In many other gastrointestinal motility disorders, the enteric nervous system is intact, but for unknown reasons it is not functioning properly.

Working with a mouse model, researchers found that RB1 may be important for the proper function of the enteric nervous system. Because RB1 is best known for its critical role in preventing tumors from forming, the scientists initially intended to study RB1's importance in the development of skin cancer. However, when the researchers deleted RB1 in certain cells of mice, they found, unexpectedly, that the mice developed severe intestinal blockages that were similar in some ways to the bowel obstructions experienced by humans with Hirschsprung's disease. The researchers examined the intestines of the mice and found that the enteric nervous system was still present, but it was disorganized, and many nerve cells were much larger than those in the control mice. In particular, the nerve cells that produce nitric oxide, a factor important for muscle relaxation, were especially affected by the loss of RB1—these cells had large, irregular nuclei (the part of the cell that contains DNA) and were unable to divide. These affected nerve cells also produced too much nitric oxide, which inhibited muscular contractions and motility in the small intestines. This study suggests that the RB1 protein is important for proper function of the enteric nervous system in mice, and it may help to understand the causes of gastrointestinal motility disorders in humans.

*Fu M, Landreville S, Agapova OA…Heuckeroth RO. Retinoblastoma protein prevents enteric nervous system defects and intestinal pseudo-obstruction. J Clin Invest 123: 5152-5164, 2013.*

**Molecular Interactions Offer Clues to Congenital Form of Diarrhea:** Scientists have uncovered the molecular workings behind a rare but severe form of inherited diarrheal disease in newborns. The rare disease, known as microvillus inclusion disease (MVID), affects some individuals of European, Middle Eastern, and Navajo American Indian descent, resulting in chronic diarrhea in newborns for which there is no treatment apart from intravenous nutrition or intestinal transplant. This genetic disease is marked by reduced sodium absorption into intestinal cells thought to be caused by the loss of tiny, finger-like projections called microvilli on the inner surface of cells lining the intestine and by gaps between the cells. However, understanding of the molecular processes gone awry in this disease remained murky. Researchers used intestinal cells grown in laboratory culture and intestinal samples biopsied from Navajo patients with a particular form of MVID gene mutation to carry out their investigations of this disease. By examining the samples from patients, they identified defects in intestinal structure and organization that occur with this disease, and they then used the laboratory-grown intestinal cells to investigate the underlying molecular mechanisms. For these studies, they genetically altered the intestinal cells to reduce production of a protein called myosin Vb (MYO5B), mimicking the mutation found in the Navajo patients with MVID. MYO5B acts as a molecular "motor," helping move other proteins within the cell to where they need to go. In both the cultured cells and patient samples, the reduction in functional MYO5B resulted in a loss of the microvilli, altered the levels of proteins that are normally in the junctions between the cells, and misdirected several proteins within the cell. Two of these proteins, RAB8A and RAB11A, were shown to play an important role, working together with MYO5B to maintain the microvilli. The loss of interactions among these proteins led to changes to the microvilli and distribution of cellular contents, characteristic of

MVID. These changes in turn profoundly affected the absorption and transport of items such as sodium, which typically helps to balance the movement of other electrolytes and fluids in and out of cells. These studies shed light on the complex molecular interactions in intestinal cells that are derailed by the mutations causing MVID, leading to severe diarrhea. These molecules and their functions may provide targets in the future for treating this severe diarrheal disease in susceptible newborns.

*Knowles BC, Roland JT, Krishnan M,...Shub MD. Myosin Vb uncoupling from RAB8A and RAB11A elicits microvillus inclusion disease. J Clin Invest 124: 2947-2962, 2014.*

**Endoscopic Procedure for Sphincter of Oddi Dysfunction Fails To Reduce Abdominal Pain:** In a clinical trial to examine a procedure used in clinical practice with the intent of relieving pain after gallbladder removal, researchers have found that this procedure, which carries considerable risk, may not be effective. The gallbladder is often removed to treat conditions such as chronic gallstones, local inflammation, or pain that is suspected to originate in the gallbladder or bile ducts. Patients occasionally experience recurrent abdominal pain after this surgery, but in many cases the source of the pain is not clearly established. One suspect has been a condition called sphincter of Oddi dysfunction (SOD). Although not proven, it has been suggested that this condition is caused when the sphincter (or circular muscle) that allows bile and pancreatic juices to flow into the intestine does not relax properly. To remedy SOD, patients typically undergo a procedure called sphincterotomy, in which a tube with a small camera is inserted through the mouth and into the intestine, and the sphincter is cut open. Sometimes an additional procedure is carried out to measure pressure in the sphincter. However, the suggested benefits of these procedures are controversial, and they carry a substantial risk of significant complications, including pancreatitis or perforation of the bowel wall.

In an attempt to address the uncertainty surrounding the treatment of SOD, a study was conducted across seven clinical centers to see if sphincterotomy actually reduced pain following gallbladder surgery. The trial included over 200 participants who experienced recurrent abdominal pain after their gallbladders were removed. The participants underwent either sphincterotomy or a mock procedure (where the camera was inserted but the sphincter was not cut) to treat their suspected SOD. In addition, pressure was measured in the sphincter using a standardized method. While both groups of participants experienced a reduction of pain severity, sphincterotomy did not reduce abdominal pain compared to the mock procedure. Additionally, between 11 and 15 percent of the participants developed pancreatitis after these procedures, underscoring the risk of complications that may occur as a result of the invasive operations. Furthermore, the sphincter pressure measurements had no correlation with the outcomes, which calls into question the idea that high pressure in the sphincter is the cause of symptoms in these patients. The results of this trial suggest that sphincterotomy does not improve pain in cases of suspected SOD following gallbladder removal—information that could save patients from the burden of this unnecessary and risky procedure.

*Cotton PB, Durkalski V, Romagnuolo J,...Robuck P. Effect of endoscopic sphincterotomy for suspected sphincter of Oddi dysfunction on pain-related disability following cholecystectomy: the EPISOD randomized clinical trial. JAMA 311: 2101-2109, 2014.*

**Complex Factors Influence Experience of Irritable Bowel Syndrome:** Two analyses of data from a clinical study on irritable bowel syndrome (IBS) outcomes point to several key determinants of quality of life and feeling healthy in individuals affected by this syndrome. IBS is a type of functional gastrointestinal disorder marked by abdominal pain, as well as diarrhea, constipation, or both; it more frequently affects women than men. The NIDDK-sponsored Irritable Bowel Syndrome Outcome Study is a multi-center, placebo-controlled randomized clinical trial with the goal of determining whether self-administered cognitive behavioral therapy is as helpful as standard therapy with a therapist in reducing IBS symptoms and overall burden. In addition to this primary aim, researchers

have used the data generated by the study to understand the impact of specific factors on the experience of women and men living with IBS.

Recognizing the strong mind-body connection thought to underlie conditions such as IBS, researchers investigated how even the anticipatory fear of IBS symptoms might affect the quality of life experienced by people with moderate to severe IBS. They administered multiple surveys to 234 study participants (nearly 80 percent of whom were women), including those designed to measure fear of future gastrointestinal symptoms, IBS symptom severity, and quality of life. Then, they quantified and analyzed the responses statistically to determine associations between these self-reported measures. The fear of IBS symptoms had a large impact on reducing individuals' day-to-day quality of life, even more so than the symptoms themselves. This finding suggests that greater attention to the major role that fear of future IBS symptoms plays in quality of life for a person with this disorder can help health care providers provide more effective care.

Another analysis of data from the study focused on the relationship between several psychosocial or physical factors and how highly people with IBS rated their health—considered an accurate predictor of their future health outcomes, such as disability or health care usage. Participants rated their health on a scale ranging from poor to excellent and simultaneously completed surveys to measure a range of possible contributors, including their IBS symptom severity, quality of life, abdominal pain, fatigue, stress, depression, anxiety, negative interactions, and social support. Their responses were quantified and analyzed statistically to identify associations. The researchers found that factors such as stress, depression, and anxiety were associated with a perception of being in worse health in those with IBS. Surprisingly, as with the other analysis, the severity of IBS symptoms played a lesser role in participants' self-assessments of their overall health. This analysis captures some of the complexity that factors into how individuals with IBS perceive their own health. Physician awareness of this complexity could help to improve the doctor-patient relationship, as well as patient satisfaction and compliance with medical care.

*Lackner JM, Gudleski GD, Ma CX, Dewanwala A, Naliboff B; Representing the IBSOS Outcome Study Research Group. Fear of GI symptoms has an important impact on quality of life in patients with moderate-to-severe IBS. Am J Gastroenterol 109: 1815-1823, 2014.*

*Lackner JM, Gudleski GD, Thakur ER, Stewart TJ, Iacobucci GJ, and Spiegel BMR; Representing the IBS Outcome Study Research Group. The impact of physical complaints, social environment, and psychological functioning on IBS patients' health perceptions: looking beyond GI symptom severity. Am J Gastroenterol 109: 224-233, 2014.*

# *Chance and Prepared Minds Lead from Lab to New Drug Development*

Science rarely moves in a straight line—like a good page-turner, the story of scientific discovery is often full of twists and turns, dead ends and red herrings, and then a sudden burst of insight, sometimes from an unexpected source. For all the painstakingly prepared proposals and long hours spent at the lab bench or clinic, scientists often speak of serendipity as playing an essential part in their tales of scientific discovery. And these discoveries, originally directed at a specific biomedical question, can at times inspire an answer to another, seemingly unrelated problem. One story with all of these elements of surprise is that of an unexpected scientific journey spanning more than 2 decades. It begins with a basic research discovery in adult intestinal cells and arrives those decades later at new opportunities for improved treatments against diseases at multiple sites throughout the human body, including two recently approved drugs for hemophilia.

**From Failure, Fortune**

On July 22nd, 1992, NIDDK grantee Dr. Richard Blumberg was working in his lab when his postdoctoral fellow came to express frustration over a failed negative control in one of his experiments. The fellow had been conducting experiments in an adult human intestinal cell line with immune molecules called antibodies, which attach to proteins on the cell surface. His negative control antibody was designed not to bind the cellular proteins that the experimental antibody was binding. But the control antibody had latched on instead to some unknown protein.

"Chance favors only the prepared mind," is a saying attributed to the microbiologist Louis Pasteur. When Dr. Blumberg looked at the result, in an instant his mind returned to his own postdoctoral training, working with a type of protein on cell surfaces called a major histocompatibility complex (MHC) class I molecule. MHC class I molecules are displayed on the cell surface to help the immune system distinguish healthy from infected cells. He experienced a flash of recognition—the control antibody was likely attaching itself to a related molecule known as the neonatal Fc receptor, or "FcRn." But, as the name of the molecule suggests, at the time it had only been found in newborn rodents, not in their adult cells, and the molecule had never been found in humans.

First discovered in the mid-1960s, FcRn was known to interact with a portion of the immunoglobulin G (IgG) antibody called the Fc domain, controlling transport of IgG across intestinal epithelial cell layers in early life in rodents. IgG is the most abundant type of antibody in the blood and extracellular spaces in internal tissues, including portions of the intestines, where it helps protect against infection. Newborn rodents receive IgG mainly from their mothers' milk *via* the aforementioned process, while in humans, IgG is transferred from mother to fetus across the placenta to confer protection.

In the years following that "failed" experiment in 1992, Dr. Blumberg's group and others confirmed that the "neonatal" Fc receptor was indeed a misnomer. The receptor continues to be produced into adulthood in a number of cell types throughout the human body. In the 1990s, they published their findings confirming FcRn in adult liver hepatocytes and intestinal epithelial cells, followed by reports in lung epithelial cells, endothelial cells that line blood vessels, and, most importantly, multiple types of cells that are involved in immunity.

They and others also uncovered a broad range of functions carried out by FcRn in humans, including carrying IgG across the placenta, transporting IgG back and forth across mucosal layers such as the intestinal and lung epithelial barriers, delivering IgG bound to a protein from an invading pathogen to alert local immune cells, and controlling the movement of IgG molecules in the circulation. The interaction between

FcRn and IgG was found to give the antibody greater stability by delaying its degradation within cells and recycling it back into the circulation. This explains why IgG lasts so much longer in the bloodstream than other proteins—for weeks rather than days or even minutes.

**Translating Laboratory Successes into Clinical Solutions**

For more than 2 decades, the NIDDK has supported Dr. Blumberg's research investigating and translating that important initial discovery of FcRn in adult human cells. A pilot grant from the NIDDK-supported Harvard Digestive Diseases Center enabled the lab's first experiments to pursue this finding in the early 1990s, collecting evidence confirming FcRn's presence and function in different human cell types. After that, the group had enough data assembled to successfully apply for an R01 grant awarded through the NIDDK in 1997, allowing them to delve more deeply into the immunology and cell biology of FcRn's functions in transporting IgG.

From that first moment of discovery in 1992, Dr. Blumberg recognized the translational potential of the FcRn-IgG system in adult humans. This system had the unique ability to move large molecules across mucosal layers in the intestine and lung, a property that would theoretically enable oral or inhaled delivery of drugs that would otherwise be deliverable only by injection. They started filing for a patent, which was issued in 1995.

The finding in 1996 by other groups of FcRn's role in recycling IgG in the bloodstream further deepened their interest in this system for improving drug delivery. By tethering large macromolecules, such as drugs, just to the Fc piece of IgG, which would then be transported through the body to their site of action and repeatedly recycled by the FcRn, they could achieve longer-acting drugs. An added benefit discovered by another group was that, because the body recognizes and tolerates the Fc portion of the IgG antibody, molecules fused to Fc were less likely to set off an adverse reaction by the immune system and thus would presumably be safer to use.

In 1999, Dr. Blumberg and others launched a new pharmaceutical company to pursue translation of this work. Along the way, Dr. Blumberg collaborated with many other "prepared minds" who collectively helped fuel further discoveries and move the science forward to translation in the clinic. They included his co-investigator on the R01 grant, Dr. Wayne Lencer, who brought expertise in cell biology; Dr. Blumberg's brother, businessman and physician Dr. Laurence Blumberg, who helped with the business plan and funding for their fledgling company; Dr. Tom Maniatis, a molecular biologist and cofounder of other pharmaceutical companies, who helped Dr. Blumberg and his colleagues to develop the new company; and others in academia and industry.

In 2004, Dr. Blumberg and his collaborators at the newly formed company published another important discovery. They were able to create a unique Fc fusion protein with erythropoietin (EPO), a naturally occurring hormone sometimes used to treat anemia, which could be delivered in aerosol form through a tube in the lungs of a pre-clinical animal model in non-human primates. They found that, by linking only one EPO molecule to two Fc domains, rather than the two molecules used in the past, the resulting fusion protein, which they called a "monomeric" Fc fusion protein, delivered by aerosol was longer-acting and more effective, similar to EPO injections in humans.

The team decided to focus their attention next on hemophilia, due to a pressing need for more longer-acting drugs. Adults and children with hemophilia A or B are deficient in a specific clotting factor in the blood, either Factor VIII or IX, respectively, which puts them at risk for bleeding episodes. However, replacing these factors was no easy matter. Due to their large size and short half-lives in the circulation, they were difficult to deliver in a form that was easy to use. Moreover, existing drugs required frequent intravenous infusions as needed, at least every few days, to prevent complications from bleeding episodes, such as severe bruising and bleeding into joints that sometimes leaves individuals crippled. This is especially problematic for children, limiting the use of these factors during one of the most vulnerable periods

of life. Further, these factors also sometimes elicited a harmful immune reaction in patients, though another group had recently shown how IgG-accompanied Factor VIII did not elicit such a response.

In 2007, the company that Dr. Blumberg helped found was sold to a larger pharmaceutical company, which used the Fc fusion technology and knowledge generated by Dr. Blumberg and colleagues to develop two long-acting Fc-fusion Factor VIII and Factor IX therapeutic agents for hemophilia A and B, respectively. Like the Fc-fusion EPO drug for anemia, these agents were designed with only one drug molecule attached to two Fc domains for greater staying power and effectiveness. The company performed clinical trials on these drugs, showing they were safe and effective. In 2014, these drugs were approved by the U.S. Food and Drug Administration (FDA), with Alprolix™ released as a hemophilia B treatment in March followed by Eloctate™ for hemophilia A in June. Eloctate™ allows patients with hemophilia A to go 3 to 5 days between infusions, while Alprolix™ extends the time between treatments even longer, up to 1 to 2 weeks.

**Bright Horizons for Better Treatments**

In addition to the hemophilia drugs based on work by Dr. Blumberg and colleagues, Fc-fusion proteins developed by other groups have been approved by the FDA since the 1990s for the treatment of other diseases, largely autoimmune in nature, such as rheumatoid arthritis and psoriasis. Antibody-based therapeutics that depend on FcRn-based biology have also shown promise against a host of other diseases, including inflammatory bowel disease, colorectal cancer, and even protection against infectious diseases such as HIV-AIDS. These versatile proteins might also be tested for other uses, including as an antidote for an adverse drug reaction and as a means to clear radioactive materials administered for imaging.

Although the hemophilia drugs based on Dr. Blumberg's and others' work are delivered by injection, Fc-fusion drugs have the potential for less-invasive delivery in the future based on their unique ability to interact with the FcRn and cross mucosal barriers like the lung and intestine. For example, patients might one day use an inhaler to deliver an Fc-fusion drug through the lung epithelial tissue to reach other disease sites throughout the body. This concept has been enabled by the team Dr. Blumberg and his colleagues assembled, as shown by successful completion of a phase I study of an inhaled Fc-fusion protein containing EPO. Researchers who have been inspired by Dr. Blumberg's work and with whom Dr. Blumberg has collaborated are also looking into using Fc fragment-coated nanoparticles as a vehicle for oral delivery of drugs that are currently administered by injection, such as insulin for diabetes, thereby improving patient comfort and compliance.

Research grants to Dr. Blumberg's group and others are continuing to support exploration of the basic biology of the Fc system and its yet-unknown discoveries, which could lead to additional clinical technologies and therapies. All in all, the future looks bright for one "failed" laboratory experiment to continue to yield fruits that benefit patients for years to come.

## PANCREATITIS RESEARCH

**Inflammatory Protein Linked to Pancreatitis:** New research has found that a protein produced by pancreatic and immune cells has a role in the development of pancreatitis in mice. The pancreas is a small organ (6 inches long in humans) that is located behind the stomach and has many vital functions, including the generation of digestive enzymes. Typically, these harsh, powerful enzymes are inactive until they leave the pancreas and enter the digestive tract. In pancreatitis, however, the enzymes are activated prematurely, damaging the pancreas and triggering inflammation. This condition is extremely painful and can lead to serious complications such as organ failure. The progression of pancreatitis is very complicated, involving many interactions between the cells in the pancreas and the components of the immune system, and many of the factors involved are not known.

While investigating factors potentially involved in pancreatitis, a group of researchers focused on a protein called interleukin-33 (IL-33). IL-33 had not been clearly linked to pancreatitis previously but was known to be involved in several other inflammatory diseases. Using male mice as a model, the scientists found high levels of IL-33 in the pancreases of the mice when pancreatitis was induced. Much of the IL-33 appeared to originate from immune cells that had migrated into the pancreas during the onset of inflammation. To see if pancreatic cells themselves could produce IL-33 during inflammation, the pancreatic cells were removed from healthy mice and exposed to a protein that typically causes inflammation. Not only did the stimulated pancreatic cells produce IL-33, but they also reacted to IL-33 by secreting additional inflammatory proteins that attract immune cells. This means that the production of IL-33 by pancreatic cells could intensify the inflammation that occurs during pancreatitis. In support of this model, the scientists found that injection of IL-33 into healthy mice caused inflammation in the pancreas, including the migration of immune cells into the organ and the release of inflammatory factors into the bloodstream. This study implicates IL-33 in the development of pancreatitis, and it suggests that controlling IL-33 levels in the pancreas could be a plausible approach in treating this condition.

*Kempuraj D, Twait EC, Williard DE, Yuan Z, Meyerholz DK, and Samuel I. The novel cytokine interleukin-33 activates acinar cell proinflammatory pathways and induces acute pancreatic inflammation in mice. PLoS ONE 8: e56866, 2013.*

## UNDERSTANDING AND TREATING LIVER INJURY

**Animal Model with "Humanized" Liver Predicts Drug Toxicity in Human Livers:** Scientists have enlisted a special type of mouse with human cells in its liver for a proof-of-concept study to predict which experimental drugs can cause liver failure and should thus not be tested in humans. As the primary spot for drug metabolism, the liver is particularly susceptible to injury from some drugs, which can result in liver failure, need for a transplant, and death. Animals such as mice are often used to test experimental drugs for any dangerous side effects before these drugs are tested in clinical trials. However, these animal models feature key physiological differences from humans that can cause a drug to be benign in the animals, yet harmful in humans. For example, in 1993, a clinical trial of a drug called fialuridine resulted in liver failure in 7 of the 15 participants, despite no indications the drug could cause liver problems when tested earlier in multiple animal models. In the recent study, a group of scientists sought a better way to test drugs for liver toxicity in the laboratory. To do this, they used "chimeric" mice whose own liver cells were mostly replaced with liver cells from human donors. Both male and female mice, as well as liver cells from both female and male donors, were used. The scientists proceeded to determine whether they could detect signs of drug-induced liver toxicity in the human, but not rodent, cells. Specifically, they tested whether this model could have been used to predict the human-specific liver failure caused by the drug fialuridine. After 4 days of treatment with the highest dose, the chimeric mice showed signs of liver toxicity, such as elevated liver enzymes, fatty liver, and cellular changes, while the control mice without any human cells did not. They then treated both the chimeric and

control mice with another drug known not to cause liver failure in humans; the lack of toxic effects showed the model could distinguish drugs that cause human liver failure from those that do not. This study demonstrates the utility of this kind of animal model with a humanized liver for screening experimental drugs. Use of these pre-clinical animal models could reduce the chances of exposing volunteers in clinical trials to experimental drugs that can cause acute liver failure.

*Xu D, Nishimura T, Nishimura S,...Peltz G. Fialuridine induces acute liver failure in chimeric TK-NOG mice: a model for detecting hepatic drug toxicity prior to human testing. PLoS Med 11: e1001628, 2014.*

**Liver Regeneration Breakthrough Using Mature Human Cells:** A team of scientists has succeeded in coaxing mature human cells to be reprogrammed into a type of liver cell that can repopulate the organ after liver failure in a mouse model. Researchers have been searching for cell-based alternatives to liver transplantation because of the limited supply of donor organs. It would be ideal to use cells from an individual's other healthy tissues to repopulate the diseased liver, but mature cells are usually terminally committed in their specific roles and cannot switch to performing the part of a liver cell. On the other hand, so-called induced pluripotent stem cells, created by reprogramming mature cells all the way back to a stem-cell-like state, have shown promise. These cells, however, have been unable to proliferate adequately *in vivo* and can also carry a risk of producing tumors.

Now researchers seem to have hit on a unique solution using cells that are not too stem-cell-like and also not too mature. Starting with mature cells called fibroblasts taken from humans, they induced the cells to regress somewhat by inserting genes for three factors produced by embryonic stem cells. They then added growth factors and other molecules to encourage the cells to take on a more liver-like persona. The cells then produced several proteins such as albumin that are distinctive for liver cells, took on a liver-cell-like shape, and displayed liver-associated functions such as storage of glycogen (a reserve form of glucose [sugar]), storage and uptake of fats, and production of urea. But the real test came when the scientists transplanted these liver-like cells into a mouse model of liver injury and failure. Over the ensuing months, the cells expanded to repopulate the liver and underwent further maturation, improving the survival of the mice. The transplanted cells also showed a lasting effect, synthesizing human albumin that was still detectable in the mouse's blood 9 months after transplant. Though further experiments are needed before this cell technology can be applied to humans, these experiments represent a major step towards using cells from a person's own body to heal their diseased liver. This technology could overcome the current challenges confronted by patients of long wait times for donor organs and life-long immunosuppressive drugs to prevent organ rejection.

*Zhu S, Rezvani M, Harbell J,...Ding S. Mouse liver repopulation with hepatocytes generated from human fibroblasts. Nature 508: 93-97, 2014.*

## HEPATITIS C PREVENTION

**Exposure to Low-level Hepatitis C Virus Does Not Protect Against Future Infection:** Scientists at the NIDDK have turned conventional wisdom—that low-level exposure to hepatitis C virus (HCV) protects against subsequent, full-strength encounters with the virus—on its head, finding instead that, in an animal model, such exposures may put individuals at risk for future infection by suppressing immune function. Researchers had observed that some people resist developing hepatitis C despite coming into contact with repeated, low doses of the virus, such as through infected family members or injection drug use; these individuals often produce a type of immune cell, called a T cell, that responds specifically to HCV. This observation led to the assumption that their immune systems were primed to protect them against a future, full-blown HCV exposure, similar to how individuals who spontaneously clear an acute HCV infection are able to quickly clear any future infections. The NIDDK team set out to test this assumption. The scientists conducted their HCV study in chimpanzees, chosen for their similarity to humans in responding to HCV, which does not infect other animal models. They gave three

chimpanzees repeated infusions of plasma and blood cells taken from people who had antibodies to HCV, but only trace amounts of viral RNA—signifying a prior, low-level exposure—and who did not show signs of an active hepatitis C infection. The chimpanzees developed a similar profile to their human donor counterparts in that they remained free of measurable levels of circulating virus, but started producing T cells that target HCV. When the researchers then challenged the low HCV-exposed animals with a full dose of the virus, the prior low-dose exposures did not protect against a full-blown viral infection. Looking more closely at certain types of T cells produced by the pre-exposed chimpanzees, they saw that these cells were ineffective at responding to a full-dose HCV infection, compared to cells from chimpanzees who were either infected for the first time or re-infected after recovering from a full-blown infection. Upon further investigation, the researchers found boosted levels of another type of T cell, called the regulatory T cell, in the pre-exposed chimpanzees. These regulatory T cells appeared to be the cause of the immune suppression toward HCV in these animals. These observations from a small group of the most closely related species to humans—chimpanzees—now await confirmation in people exposed to repeated, low doses of HCV. However, they provide strong evidence that runs contrary to the belief that low-level HCV exposure protects individuals against acute hepatitis C. This finding is relevant to designing effective vaccination strategies in the future against HCV and other microbes to which humans can be repeatedly exposed, such as those that cause malaria, tuberculosis, and HIV/AIDS.

*Park SH, Veerapu NS, Shin EC,…Rehermann B. Subinfectious hepatitis C virus exposures suppress T cell responses against subsequent acute infection. Nat Med 19: 1638-1642, 2013.*

# The Light and Dark Sides of an Intestinal Heat Shock Protein

*Dr. Eugene B. Chang*

*Dr. Eugene B. Chang is the Martin Boyer Professor of Medicine at the University of Chicago. A graduate of The Johns Hopkins University, he earned his medical degree from the University of Chicago School of Medicine, where he has been a member of the faculty since 1986. He presently has an NIDDK grant that supports training for post-doctoral researchers in metabolism and nutrition, and he serves as a co-director of the Pritzker School of Medicine Summer Research Program. His research focuses on host-microbial interactions in the intestines, particularly in defining signal pathways that are involved in maintaining intestinal stability. His studies are also aimed to better understand how perturbations of gut bacteria contribute to the development of digestive diseases, especially inflammatory bowel disease. He has defined several new mechanisms of action of probiotic organisms that are currently being developed as therapeutic agents. He joined the National Diabetes and Digestive and Kidney Diseases Advisory Council in 2014. At the Council's May 2014 meeting, Dr. Chang presented a lecture on the multiple roles of a heat shock protein in digestive diseases.*

The gastrointestinal (GI) tract is one of the harshest environments in the body. The cells that line the stomach are continuously bathed in acids and digestive enzymes. At the other end of the tract, the cells that line the colon are exposed to millions of bacteria. These microbes must be kept in check to prevent inappropriate inflammatory reactions that could lead to inflammatory bowel disease (IBD)—a distressing and serious condition that causes persistent diarrhea, cramping abdominal pain, fever, and rectal bleeding. In some cases, the symptoms of IBD can only be alleviated by surgical removal of the colon, followed by a lifelong need for an external ostomy pouch to collect intestinal contents. In other cases, people with IBD may eventually develop colon cancer.

One focus of Dr. Chang's research is to gain further understanding of IBD by studying how the cells lining the GI tract respond—either appropriately or inappropriately—to the bacteria in the gut. He shared results from his lab that tell an interesting story of how a type of protein called a "heat shock protein" (HSP) can protect intestinal cells from the many dangers in the GI tract. But, as Dr. Chang explained, although a certain HSP can safeguard cells from injury, it may also have a darker, dangerous side.

### Heat Shock Proteins as Guardians Against Stress

It is not surprising that HSPs are found in virtually every cell in the body, because they play an important role in protecting other proteins in the cell from many types of external stress. For example, when cells are exposed to high heat, such as during a bad fever, many proteins can lose their shapes and begin to unravel, impairing their ability to function properly. These defects in the proteins could lead to the death of the cell. However, if the temperature is raised gradually, the cells can respond by producing HSPs to rescue the damaged proteins. The HSPs interact with the impaired proteins and return

them to their original shape so that they can function normally and move to the proper part of the cell. In this way, the HSPs act as guides to allow proteins to regain (or retain) their natural, functional shape, and they help cells survive during environmental stress.

While some types of HSPs are "constitutive," or always present, others are "inducible" and only produced when the cell is exposed to stressful conditions, such as heat, infections, acids, or other factors that may cause protein damage. In fact, the amount of HSPs produced in times of cellular stress is so large that they may be 10 percent of the total protein in the cells.

Not surprisingly, these HSPs are highly induced in the turbulent environment of the gut. Dr. Chang presented evidence that HSP levels are particularly high in the stomach, where acids and digestive enzymes are plentiful, and in the colon, where the majority of gut bacteria live. The cells that have the highest amount of HSPs are also the ones that are in direct contact with these hazards.

**Hsp70: The Intestinal Cell's Response to Gut Bacteria**

There is ample evidence that intestinal cells produce HSPs in response to the exposure to gut bacteria. One type of HSP in particular—Hsp70—is produced in abundance in the area of the colon closest to the small intestine, and its presence tapers closer to the rectum. This correlates with the types of microbes that are present in those regions, Dr. Chang explained. On the other hand, the guts of germ-free mice—which do not have gut bacteria—do not contain the mouse form of Hsp70. This provides further evidence that Hsp70 production in intestinal cells is triggered by exposure of the cells to gut microbes.

Dr. Chang and his research team decided to closely examine this relationship between gut bacteria and Hsp70 by allowing gut microbes to colonize an area of a rat's gut that is typically not exposed to high levels of bacteria, and then determining whether this results in Hsp70 production. They chose the small intestine, which typically harbors far fewer bacteria than the colon. One reason for this difference is that intestinal contents tend to move much more slowly in the colon, and this sluggish motion allows bacterial colonization to take place. To see if bacterial colonization would cause Hsp70 to be produced in small intestinal cells, Dr. Chang's team decided to alter a portion of the small intestine to limit the movement of its contents, making it more like the colonic environment and encouraging bacterial growth. They accomplished this by performing surgery on male rats to create "blind loops" in their small intestines. A blind loop is a short branch of intestine with one end closed off to form a dead end. When the blind loop was oriented so that the dead end was at the bottom of the branch, intestinal bacteria became trapped there, and Hsp70 was produced in the cells within the loop. When the blind loop was oriented so that the dead end was at the top of the branch, the contents of the blind loop were pushed out by the regular muscle contractions of the intestine, and there was little to no production of Hsp70. In other words, this inducible HSP can be highly produced in portions of small intestine, but only under conditions that promote bacterial growth. This, said Dr. Chang, is a great example of how gut microbes affect the human body.

Dr. Chang next explored why Hsp70 production is triggered by gut bacteria. Because HSPs are known to have a vital role in protecting cells from stress, it is possible that they have a protective role in the gut, perhaps by helping the intestinal cells heal after they become damaged by inflammatory diseases like Crohn's disease or ulcerative colitis. To test this idea,

# SCIENTIFIC PRESENTATION

Dr. Chang's team gave mice a chemical called dextran sodium sulfate (DSS), which is used experimentally to cause colitis, or inflammation in the colon. In normal mice, DSS caused acute (brief) colitis that healed completely 10 to 14 days later. However, in mice that could not make Hsp70, the injury caused by DSS was more severe and led to a chronic (long-lasting) form of colitis that persisted even after withdrawal of DSS. The colons from the mice with this chronic colitis were similar to those in humans who have ulcerative colitis, a form of IBD that specifically affects the colon. This suggests that Hsp70 is important for returning the colon to a healthy state after it has been damaged—which, Dr. Chang explained, is the "good" side of Hsp70.

**The Dark Side of Hsp70**

One of the dangers of chronic colitis is that it may eventually lead to colon cancer. In humans, most cases of colon cancer—called "sporadic" colon cancer—first develop as polyps that protrude from the wall of the colon. The type of colon cancer that arises from colitis is different, however; it develops from multiple flat lesions instead of bulging polyps. When mice are exposed to DSS in combination with a cancer-promoting agent, they eventually develop cancerous polyps similar to those in sporadic colon cancer. However, when Hsp70 is absent in these mice, colitis leads to the development of flat tumors that are remarkably similar to the tumors in the human form of colon cancer that arises from colitis. This means Hsp70 determines what type of colon cancer will grow in mice with colitis.

But there is an even darker side to Hsp70, Dr. Chang explained. While Hsp70 is absent in the flat tumors of human IBD-associated colon cancer, the level of Hsp70 is extremely high in sporadic colon cancer. This means that Hsp70 may not just influence the type of cancer that grows, but could also promote the growth of sporadic tumors. To test this possibility, Dr. Chang's team used a mouse model of sporadic colon cancer that will spontaneously develop colon polyps. When the levels of Hsp70 were high in these mice, there was a drastic increase in the size and number of tumors; when Hsp70 was deleted from these mice, the tumors were smaller and less abundant. These results suggest that Hsp70—the same protein that helps intestinal cells survive damage from IBD—also contributes to sporadic tumor formation and progression in the colon.

**BAGging Beta Catenin: How Hsp70 Encourages Tumor Growth**

Dr. Chang next explored how Hsp70 may help drive the development of sporadic colon cancer. One possibility was that Hsp70 was somehow influencing the activity of a protein called beta catenin, which is a primary player in many cases of colon cancer. Beta catenin is usually found outside the cell's nucleus (the part of the cell that contains DNA), but when it is active, it will move into the nucleus, where it turns on genes that can help cancer cells grow and spread to other parts of the body. Not surprisingly, beta catenin is found primarily in the nuclei of tumor cells in cases of sporadic colon cancer. However, in mouse tumor cells that lack Hsp70, beta catenin is found primarily outside the nucleus. This means Hsp70 is somehow helping beta catenin move to the nucleus where it can encourage tumor cells to grow and invade other tissues.

The next step was to find out exactly how Hsp70 was directing beta catenin. One clue came when Dr. Chang's team discovered that Hsp70 actually associates with beta catenin and accompanies it into the nucleus. Additional experiments identified another molecule, called BAG-1, that also associates with Hsp70, resulting in an aggregate of Hsp70, BAG-1, and beta catenin. Importantly, BAG-1 contains a built-in signal that directs

it into the nucleus, taking Hsp70 and beta catenin with it. This suggests that, through its interactions with BAG-1, Hsp70 acts as a "chaperone" to usher beta catenin into the nucleus where it can activate genes that promote tumor growth and dissemination.

**Future Directions**

Dr. Chang closed by thanking his research team. His exploration of Hsp70 paints a fascinating yet disquieting picture of a protein that not only protects intestinal cells from stress, but also can, under certain conditions, contribute to the formation and growth of sporadic tumors in the colon. This study also illustrates the intricate relationships among colon cancer, gut bacteria, and digestive diseases such as inflammatory bowel disease. By knowing how diseases progress in the GI tract and identifying the factors that are involved—such as Hsp70—new therapies can be developed to treat or manage these conditions.

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## Tarrie Barnes

*Putting an End to a History of Hepatitis C*

Tarrie Barnes

Tarrie Barnes was 12 or 13 years old when, on the way home from church, she and her siblings walked by the Baltimore hospital where her grandmother was staying. The image of her grandmother waving to her from the window holds a special place in Tarrie's memory. "It was a sunny day," she remembers. "And when I think of my grandmother, I think of happiness."

Although she was too young at the time to comprehend exactly why her grandmother was in the hospital, Tarrie would learn years later about the disease that eventually took her grandmother's life. "I just happened to be reading her death certificate, and it said 'cirrhosis of the liver,'" she recalls. "And I thought, well, my grandmother didn't drink, so why would it say 'cirrhosis'?" When Tarrie asked her grandmother's doctor about the death certificate, he told her that her grandmother's liver had succumbed to a disease called, at the time, "non-A, non-B hepatitis."

For Tarrie, the diagnosis was a premonition of her own future struggles with a silent yet debilitating and potentially fatal liver disease.

**"Something Doesn't Look Right in Your Blood"**

Tarrie, who is now 65, had been very close to her grandmother. As a child she would rather join her family at her grandparents' home on Saturdays instead of going to a park to play. At the house, festivities would begin: guitars, harmonicas, and food. Her grandmother was a first-rate cook—she would "dip her finger in something and make it taste good"—and would always greet Tarrie and her siblings by offering them something to eat. Her grandfather would proudly stand with his family and "put his fingers under his suspenders and bounce on his heels, and say, 'Look at what I started. I started all of this,'" Tarrie fondly reminisces. She treasures the memories of those Saturdays with her grandparents. "I just feel blessed that I was in the family I was in. And anytime we saw my grandmother smile, it made the day even nicer."

Tarrie's grandmother died in 1988. A year later, scientists published reports identifying a new virus, the hepatitis C virus, as the cause of non-A, non-B hepatitis.

Then, in 1990, after she had donated blood, Tarrie received a troubling letter from the American Red Cross. The letter, she remembers, essentially said: "Something doesn't look right in your blood." The hepatitis C virus—the same virus that had stricken Tarrie's grandmother—was suspected to spread through blood transfusions, so the Red Cross had begun to screen their supply for

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infected blood. Also unsettling for Tarrie was that 15 years earlier, well before the screening had started, she herself had received a blood transfusion during surgery, meaning that she may have been exposed to the hepatitis C virus.

While the Red Cross letter stated that Tarrie could have viral hepatitis, it also mentioned the chance of a "false positive," which meant there was a possibility she wasn't infected even though she tested positive. (At this time, the screening methods were not as accurate as they would be a few years later.) Nevertheless, the Red Cross recommended that she have her blood checked, so Tarrie went to her doctor to get tested. The results came back negative. "I never thought anything else about it," remembers Tarrie. "I thought [the original Red Cross test] was a false positive." So Tarrie went back to her career at a telecommunications company and life with her husband and two children. But, at her doctor's recommendation, she stopped donating blood.

*Tarrie was slowly getting tired more easily—something that, understandably, many people could experience without raising alarm. "Some days I would feel more tired than others....You don't realize that something is going on."*

Nine years would pass. In the meantime, Tarrie started taking classes to fulfill her dream of becoming a teacher. But she also slowly began to experience symptoms that she casually attributed to aging, like many people would. She had occasional dizzy spells, sometimes to a point where she needed to hold on to her chair to keep the room from spinning. And sometimes she felt a pain in her side when she lifted something heavy. Tarrie didn't realize she had liver disease; she remembers thinking that "it was maybe my blood pressure." Most of all, she was slowly getting tired more easily—something that, understandably, many people could experience without raising alarm. "Some days I would feel more tired than others. Sometimes I couldn't do all that I wanted to do. I would get tired without knowing I was tired, because you're just used to it. You don't realize that something is going on."

It is common for people with hepatitis C not to realize that they have the disease. In fact, most people do not have any symptoms until the virus causes significant liver damage, which could ultimately result in the need for a liver transplant. Prior to the discovery of the virus and routine screening of the blood supply, many people acquired hepatitis C through a blood transfusion—the virus is most commonly transmitted by its introduction directly into the bloodstream. Once in the blood, the virus then infects cells in the liver, slowly killing them and causing scar tissue to form. Exposure to infected blood usually results in a chronic (long-lasting) infection because the body cannot get rid of the virus.

For Tarrie, the diagnosis did not come until she went to her doctor for a routine checkup in 1999. Her doctor told her that her liver test results were abnormal, and it was recommended that she see a liver specialist—the same specialist, coincidentally, who had treated her grandmother. Still not realizing she was sick, Tarrie's big shock came when the specialist walked into the examination room: "We're not going to talk about a liver transplant" were the first words out of his mouth. Taken aback, Tarrie began to realize that she could be dealing with something serious.

By 1999, there was a more accurate test for hepatitis C, and Tarrie tested positive. After a liver exam, she was diagnosed with advanced hepatitis, which means her

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liver was damaged so badly by the virus that it was beginning to scar and lose functionality. If unchecked, the disease would ultimately cause her liver to fail.

**Managing Life with Hepatitis C**

Armed with advice from her liver specialist, and knowing how the disease affected her grandmother, Tarrie began her fight against hepatitis C. Her liver specialist encouraged her to join a hepatitis C support group that he supervised, and she began to learn as much as she could. "My mother believed in education and reading," says Tarrie. "She always made sure we read. I inherited that bug." The support group was a diverse assembly of people with hepatitis C who shared their experiences and learned from each other's struggles and successes. They discussed the symptoms they were having and how to deal with them. They talked about the changes in lifestyle they should adopt when dealing with a damaged liver, such as eating healthy and avoiding alcohol. When you have advanced hepatitis, you need to be careful, says Tarrie. "Anything you put in your body goes to your liver."

Tarrie eagerly soaked up knowledge about hepatitis C while giving encouragement to other members of the group. "By that time I was 51, and I had become more of a talker," she says. "I liked sharing. I liked learning about what was going on. And we did help each other....It was a good thing."

Tarrie's liver specialist also convinced her to undergo a 6-month clinical trial at the hospital in Baltimore. She went on medical leave from her job, because "I wasn't sure about what I would be facing," she recalls. Her support group had prepared her for potential side effects that would come along with anti-hepatitis medications. "I was told what I might experience would be similar to chemotherapy. You might lose your hair, or get chills or a fever. It affects each person in a different way."

The treatment ultimately wasn't successful for Tarrie, and her hepatitis remained at an advanced stage. But this was still only the beginning of her long, complicated battle with hepatitis C.

**The First Trials at the NIDDK**

Shortly after Tarrie's diagnosis, her daughter, then a biology major at Bowie State University, was selected for a research internship at the NIH. "She is so smart. She gets it from my mother, not me," Tarrie says glowingly of her daughter. "She loves biology."

Inspired in part by her mother's predicament, Tarrie's daughter began working in the laboratory with Dr. Theo Heller, a clinical investigator in the NIDDK Intramural Research Program, under the direction of Dr. T. Jake Liang, Chief of the NIDDK Liver Diseases Branch. She was a part of one of the first teams to successfully produce the hepatitis C virus in cultured cells, a major milestone that allowed scientists to study the life cycle of the virus more closely. She also told Dr. Heller about her mother, and he suggested that Tarrie participate in a clinical trial at the NIDDK. Dr. Heller "asked my daughter three questions about me," Tarrie remembers. "Did I join a study? Did I complete the study? Did I still have hepatitis C? The answer was 'Yes' to all three. From that, I qualified to go there."

*"I enjoy going there," says Tarrie of her visits to the Clinical Center. "Everyone there at NIH has been so nice....It's like a big happy family."*

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In 2001, Tarrie enrolled in her first NIDDK clinical trial at the NIH Clinical Center in Bethesda, Maryland. For 6 months, she took a combination of two antiviral drugs: interferon, which helps the body to defend itself against viruses, and ribavirin, which slows the replication of viruses. The treatment brought her virus levels down, and her liver began to show some signs of recovery. There were side effects, but for the most part they were manageable. "At the beginning I was in bed with chills and fever," recalls Tarrie. "Then I was just tired, but I didn't realize that the tiredness was probably more from the hepatitis."

By the time Tarrie completed the trial, the drugs had reduced the amount of virus in her blood, but they did not eliminate it. The virus continued to multiply over the next few years, and the condition of Tarrie's liver regressed. It didn't stop her from achieving her goal of becoming a teacher, however. In 2006, she graduated from Morgan State University and started teaching the first grade. But her health continued to decline, and she reluctantly decided to retire after a few years. "I didn't really have a choice," she remembers. "I could tell that confusion was starting to set in, and it was starting to interfere with my ability to teach. It was emotional … because I didn't want to leave."

Still, Tarrie was not discouraged, and she enrolled in another trial at the NIDDK, using a drug regimen similar to her last trial. However, this time one of the drugs was coated in lactose—and Tarrie didn't know she was lactose intolerant. "That trial was my worst," she recalls. "That really did me in. I was living in the bathroom." Determined nonetheless, she completed the grueling 6-month trial. But the treatment did not clear her of the virus—it soon rebounded again, and Tarrie was back at square one. Yet, she still held out hope.

Tarrie gives accolades to her faith and her family for helping her through the rough times. "Life has been an adventure," she says. "Faith has gotten me through a lot. And just having a loving family has made all the difference. It all started with my grandparents—letting us know to put God first, then family, and friends. As long as you have that love that connects you, you can get through anything."

**One More Clinical Trial at the NIDDK: Saying Farewell to Hepatitis C**

After three unsuccessful treatments for her hepatitis, Tarrie once again signed up for a clinical trial at the NIDDK. She was encouraged by breakthroughs in the understanding of the disease, and she had developed a close relationship with Dr. Heller. "He's fantastic. He told me they were always working on new and better medications. The more they learned about the virus, the better the medications they could get to help clear it."

The trial was, in fact, testing two new drugs called daclatasvir and asunaprevir that directly target specific components of the hepatitis C virus. Also, due to advances in the understanding of the virus, the staff at the NIH Clinical Center were able to identify the subtype that had infected Tarrie: it was called "genotype 1b." Because different genotypes of the hepatitis C virus can respond uniquely to different medications, knowing the genotype allows doctors to predict how successful a treatment will be. In Tarrie's case, the "b" was crucial. "I was excited when they told me that I was type b, because that meant I didn't have to take interferon [for this trial]," she explains.

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*Tarrie was encouraged by breakthroughs in the understanding of the disease: "The more they learned about the virus, the better the medications they could get to help clear it."*

Tarrie began the 6-month trial near the end of 2013. Three months after she completed the study, the virus could no longer be detected in her blood—the new drugs had worked. Tarrie was ecstatic. Moreover, the two pills she took had absolutely no negative side effects. "It was like heaven compared to the last study," laughs Tarrie. In fact, Tarrie was so thrilled by the results that she presented Dr. Heller with a challenge: if she remains clear of the virus, he will have to dance a jig for her. When Dr. Heller said he didn't know how to do the jig, Tarrie responded playfully, "Google it!"

**A New Chapter: Living Hepatitis-free**

The hepatitis C virus has now been undetectable in Tarrie's blood for over 8 months, which means the odds of the virus recurring are very low. Her dizzy spells have become less frequent. In September 2014, she flew to Hawaii to visit her son, who is a naval officer, and to celebrate her 40th wedding anniversary with her husband. She still makes visits to the NIH to have her progress monitored. "I enjoy going there," says Tarrie. "Everyone at NIH has been so nice. The first or second time I went there, they already knew my name. I would even see doctors who I didn't know, but knew of my case, and they would say 'Hi, I heard you were doing well.' So, it's great. It's like a big happy family."

Not only has Tarrie's health improved, but her participation in the trials at the NIDDK also allowed her to contribute to the ongoing research on treatments for hepatitis C. "Those two pills, I think they can help a lot of folks," she says, referring to the two medications she took during her last NIDDK trial.

Tarrie holds a deep admiration for her grandmother, the woman who always made her happy. She would love the opportunity to continue to share the same happiness with her own grandchildren—in fact, she once told her church pastor that all she wanted to do was to live as long as her grandmother did.

Thanks to Tarrie's perseverance, along with a good dose of progress in medical research, she is well on her way.